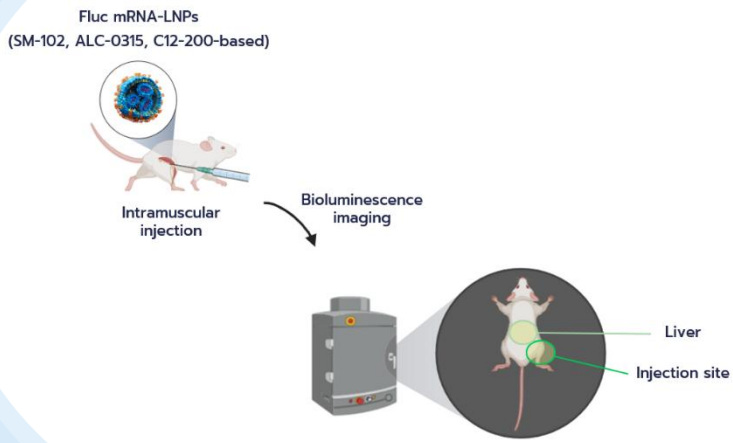
Application note Short summary

In Vivo Evaluation of Standard mRNA-LNP Formulations Produced with TAMARA

RNA-based therapeutics are transforming approaches to disease treatment and prevention, driven by their rapid design, high programmability, and non-integrating nature. However, their clinical application remains limited by instability and delivery challenges.

Lipid nanoparticles (LNPs) have emerged as a powerful platform for enabling the in vivo use of RNA therapeutics by protecting RNA from degradation and enabling its efficient intracellular delivery. Clinically validated through siRNA therapeutics as well as mRNA and saRNA vaccines, LNPs offer scalable, flexible, and efficient solutions for RNA-based applications.

In this work, **mRNA-LNPs produced using the TAMARA** microfluidic system were evaluated across **multiple standard lipid formulations, including SM-102, ALC-0315, and C12-200**. In vivo performance was assessed following intramuscular (IM) administration in mice using firefly luciferase mRNA, provided by CPI (Centre for Process Innovation, Sedgefield, UK), as a reporter.



All formulations demonstrated successful in vivo expression, with formulation-dependent differences in signal intensity and persistence.

This work was carried out at the University of Strathclyde in the laboratory of Prof. Yvonne Perrie by MSc Jade Forrester, and supported by PhD Claire Counil, PhD Audrey Nsamela and PhD Sezen Gul from Inside Therapeutics.

Physicochemical characterization of mRNA-LNPs

Firefly luciferase (Fluc) mRNA-LNPs formulated with SM-102, ALC-0315, and C12-200 were produced using TAMARA at two total flow rates (TFR 1 and 5 mL/min), and their Z-average size and polydispersity index (PDI) were measured in triplicate by DLS.

Key findings

- **Increasing TFR** from 1 to 5 mL/min led to a **decrease in particle size** across all lipid formulations, with a **slight increase in PDI** that remained within acceptable ranges.
- The **effect of TFR on size and PDI was consistent** across all lipid compositions.
- At TFR 5 mL/min (used for in vivo studies), **encapsulation efficiency (EE%) exceeded 90%** for all formulations as measured by the RiboGreen assay.

TAMARA enables reproducible LNP formulation with controlled size tuning via TFR and consistently high EE%, supporting their use in downstream in vivo applications.

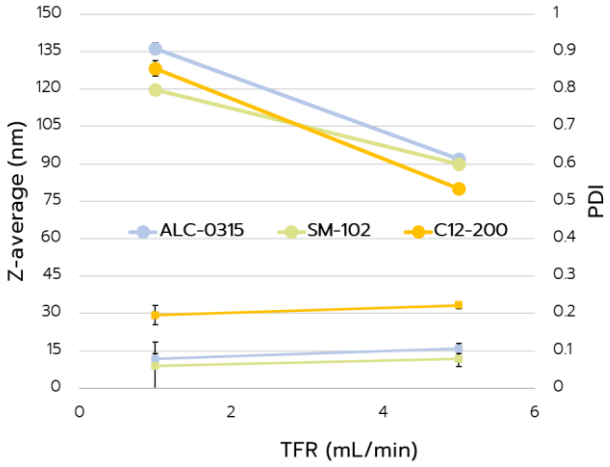
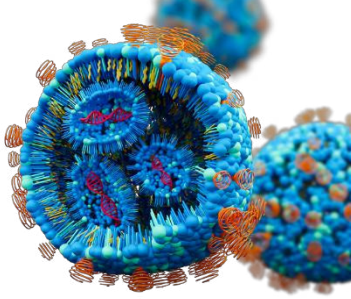


Figure 1 : Effect of TFR on LNP size and PDI for SM-102, ALC-0315, and C12-200 formulations produced using TAMARA.



In vivo expression & biodistribution of mRNA-LNPs

Fluc mRNA-LNPs formulated with SM-102, ALC-0315, and C12-200 were administered intramuscularly in mice at a dose of 2 µg mRNA per animal (1 µg per leg), and luciferase expression was monitored over time by in vivo bioluminescence imaging (ventral view), followed by quantitative analysis of total flux at the injection site and in the liver.

Key findings

- All formulations enabled **detectable in vivo expression,** confirming **successful mRNA delivery and translation**.
- Bioluminescence imaging showed strong signal at early time points (Day 0–2), followed by a progressive decrease, becoming undetectable by imaging after Day 7 due to image scaling and reduced intensity.
- Quantitative analysis revealed **formulation-dependent differences in signal intensity,** with SM-102 > ALC-0315 > C12-200 at the injection site and SM-102 ≈ ALC-0315 > C12-200 in the liver.
- Higher liver signals at early time points (Day 0–2) reflect initial systemic distribution of a fraction of the administered LNPs. Over time, liver-associated signal declined more rapidly.
- Total flux measurements confirmed a **progressive decay in signal over time**, consistent with **transient mRNA expression**.

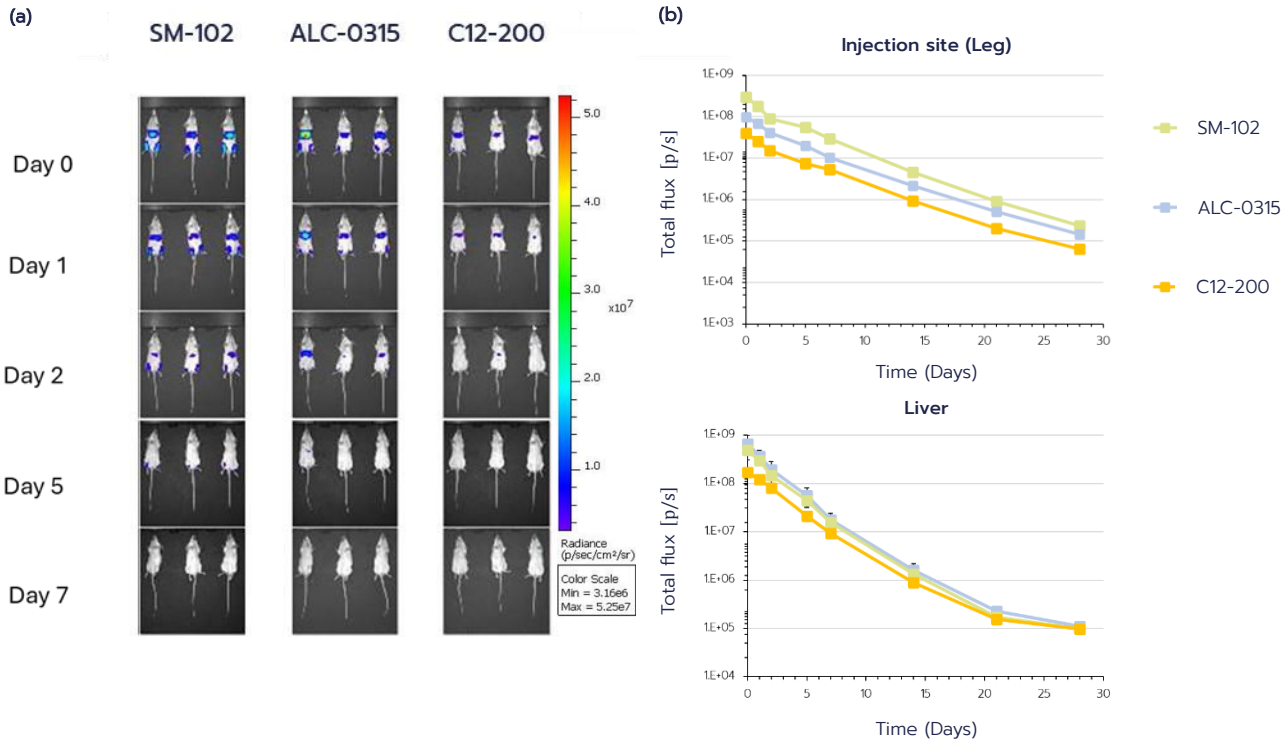


Figure 2: (a) Representative in vivo bioluminescence images (ventral view) following IM administration of Fluc mRNA-LNPs formulated with SM-102, ALC-0315, and C12-200 at selected time points (Day 0, 1, 2, 5, and 7).
(b) Quantitative analysis of total flux over time at the injection site (top) and in the liver (bottom). All time points are shown (Day 0 - 28).

TAMARA-formulated mRNA-LNPs show efficient in vivo expression with formulation-dependent performance.

Conclusion

This application note demonstrates the **in vivo performance of TAMARA-formulated mRNA-LNPs across standard lipid compositions**. SM-102, ALC-0315, and C12-200-based mRNA-LNPs:

- Maintained **controlled physicochemical properties and high EE%** suitable for in vivo use.
- Enabled **efficient and transient in vivo expression** across all formulations.
- Revealed **formulation-dependent performance**, with SM-102 > ALC-0315 > C12-200 in expression levels at the injection site.

TAMARA enables the reproducible production of mRNA-LNPs with robust in vivo expression, supporting rapid formulation screening and optimization.

Read the full Application Note on https://insidetx.com

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